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Are retinal microvascular abnormalities associated with large artery endothelial dysfunction and intima-media thickness? The Hoorn Study

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ABSTRACT

It has been hypothesized that microvascular dysfunction affects endothelial dysfunction of the large arteries, which may explain the relationship of microvascular disease with macrovascular disease. The aim of the present study was to investigate the relationship of retinal microvascular disorders with endothelium-dependent FMD (flow-mediated vasodilatation) and carotid IMT (intima-media thickness). A total of 256 participants, aged 60–85 years, 70 with normal glucose metabolism, 69 with impaired glucose metabolism and 109 with Type II diabetes, were included in this study. All participants were ophthalmologically examined, including funduscopy and two field 45° fundus photography, and were graded for retinal sclerotic vessel abnormalities and retinopathy. Retinal arteriolar and venular diameters were measured with a computer-assisted method. Brachial artery, endothelium-dependent FMD and carotid IMT were assessed ultrasonically as measurements of endothelial function and early atherosclerosis respectively. After adjustment for age, sex and glucose tolerance status, retinal vessel diameters, retinal sclerotic vessel abnormalities and retinopathy were not significantly associated with FMD. In contrast with other retinal microvascular abnormalities, retinal venular dilatation was associated with increased IMT [standardized β value (95% confidence interval), 0.14 (0.005–0.25)]. This association was attenuated and lost statistical significance after adjustment for cardiovascular risk factors, in particular after correction for fasting insulin. In the present study, retinal microvascular disorders are not independently associated with impaired FMD. In addition, retinal venular dilatation is associated with increased IMT, although non-significantly after multivariable adjustment for cardiovascular risk factors. Therefore our data provide evidence that retinal microvascular disease is of limited value in risk stratification for future cardiovascular events.

Key words: cardiovascular event, endothelial dysfunction, flow-mediated vasodilatation, intima-media thickness, microvascular disorder, retina.

Abbreviations: AVR, arteriole-to-venule ratio; BMI, body mass index; BP, blood pressure; CRAE, central retinal arteriolar equivalents; CRVE, central retinal venular equivalents; CI, confidence interval; FMD, flow-mediated vasodilatation; HbA_{1c}, glycated haemoglobin; HDL, high-density lipoprotein; HOMA, homoeostasis model assessment; IMT, intima-media thickness; NMD, nitroglycerine-mediated vasodilatation; OGTT, oral glucose tolerance test; SVC, sclerotic vessel abnormalities.

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INTRODUCTION

There is growing evidence that microvascular disease plays a prominent role in the pathogenesis of macrovascular disease and mortality [1–3]. Previous studies have shown that coronary microvascular disease may be involved in the occurrence of myocardial infarction without overt coronary artery blockage [4–7], heart failure [8] and mortality after myocardial infarction [9]. In addition, microvascular dysfunction in the skin, of which the microcirculation is thought to resemble the systemic microcirculation, has been associated with increased coronary heart disease risk [10]. Most of these studies, however, were carried out in experimental settings with small sample sizes, partly because of the invasive or laborious methods.

The retinal circulation offers a unique opportunity to non-invasively analyse the systemic microcirculation in large populations [11]. A new computer-assisted method has been developed to measure retinal vessel diameters from digitized photographs [12]. Retinal microvascular abnormalities, such as retinal arteriolar narrowing and retinopathy, have been associated with hypertension [13–17], the metabolic syndrome and Type II diabetes [18,19], and with prevalent and incident CVD (cardiovascular disease) and mortality [2,11,20–22], suggesting that retinal microvascular disorders may be a marker of atherosclerosis. In contrast, other studies have not shown an evident relationship between retinal microvascular abnormalities and markers of atherosclerosis or macrovascular disease [23–26].

If microvascular dysfunction does indeed affect the risk of atherosclerosis, the question remains through what mechanism this occurs. We hypothesized that microvascular dysfunction may be linked to atherosclerosis by inducing endothelial dysfunction of large vessels [27]. If this is the case, then markers of microvascular dysfunction should be associated with markers of large artery endothelial dysfunction and of early atherosclerosis. To test this hypothesis, we investigated cross-sectionally in a population-based study of individuals with normal glucose metabolism, impaired glucose metabolism and Type II diabetes the relationship of retinal microvascular abnormalities on one hand with brachial artery endothelium-dependent FMD (flow-mediated vasodilatation) [28,29] and carotid IMT (intima-media thickness) on the other.

METHODS

Study population

The Hoorn Study is a population-based cohort study of Type II diabetes and its cardiovascular complications among 2484 Caucasian people, aged 50–75 years, which started in 1989. Details have been described elsewhere [30]. At baseline, an age-, sex- and glucose-tolerance stratified subsample of 631 participants was extensively studied for reasons of efficiency. In 2000–2001, a follow-up examination was carried out among surviving participants who gave their permission to be re-contacted [31]. Of the 631 participants who had an ophthalmological examination at baseline, almost 60 % dropped out for the follow-up examination, because of the following reasons: 119 (19 %) persons had died, 49 (8 %) had moved out of the region and 207 (33 %) did not participate because of mobility or health problems, or lack of motivation. Finally, 256 people were included in the follow-up ophthalmological examination [17], 70 with normal glucose metabolism, 69 with impaired glucose metabolism and 109 with Type II diabetes [32]. Written informed consent was obtained from all participants. The Ethical Review Committee of the VU University Medical Center (VUmc) in Amsterdam, The Netherlands, approved the Hoorn Study.

Ophthalmological examination and retinal vessel diameter measurements

After mydriasis with 0.5 % tropicamide and 2.5 % phenylephrine eye drops, the retina was examined by funduscopy and fundus photography, as described previously [17]. Briefly, fundus photography was performed with a 45° CR5 non-mydriatic retinal camera (Canon), interfaced to a 3CCD Color Video Camera (Sony). The quality of each photo was checked immediately on a connected colour video monitor (Trinitron; Sony) and a new photograph was taken if the quality was insufficient. The photographs were digitized, compressed (10:1 JPEG), and stored on a magneto-optical disc using the MV-300P Viewfile system (TEAC). Two photographs were made of each eye, one centred on the macula and one centred on the optic disc.

Methods used to measure and summarize retinal vessel diameters from digitized photographs followed a standardized protocol [12]. Briefly, one investigator, blinded to the identity of the participant, independently measured all arterioles and venules 0.5–1 disc diameter from the optic disc margin using a computer-imaging program (Retinal Analysis; Optimate). The branches of arterioles were also measured if the trunk measurements were $\geqslant 85\ \mu$m. Computer-assisted measurements of the diameters of arterioles and venules were obtained and combined according to the revised formulas of Parr and Hubbard [12,33–35], which account for magnification differences and the number of vessels in photographs. Average diameters of arterioles [CRAE (central retinal arteriolar equivalents)] and venules [CRVE (central retinal venular equivalents)] in one eye were assessed and combined into an AVR (arteriole-to-venule ratio). An AVR of 1.0 indicates that the diameters of the arterioles are approximately equal to the diameters of the venules, whereas a smaller AVR indicates narrower arterioles or

wider venules. For each subject, one photograph centred on the optic disc was used, alternately selected from the left and right eye (1:1 ratio). In the case of insufficient quality, the photograph of the other eye was examined. Generalized arteriolar narrowing was defined as the lowest quintile of the CRAE, and generalized venular dilation as the highest quintile of the CRVE [19]. The intra-observer intersession coefficients of variation [S.D. of the mean difference/($\sqrt{2}$ × pooled mean)] were 5 % for CRVE, 8 % for CRAE and 9 % for AVR.

The fundus photographs were independently analysed by two individuals to grade retinal SVC (sclerotic vessel abnormalities) and retinopathy. In the case of disagreement, the judgment of a third investigator was taken to be decisive. Retinal SVC were defined as the presence of venous beading, focal narrowing, arteriovenous crossing changes, 'copper' or 'silver' wiring, dilated or tortuous retinal veins, or central or branch venular occlusion. Retinopathy was defined as the presence of one or more microaneurysms, haemorrhages or hard exudates, possibly in combination with areas of neovascularization, fibrous proliferation and/or laser coagulation scars in at least one eye according to the Eurodiab classification [17,36].

Endothelium-dependent and endothelium-independent vasodilatation

Endothelium-dependent FMD is largely mediated by endothelium-derived nitric oxide and is considered to reflect impaired endothelial function. The ultrasound examination was carried out according to the guidelines of the International Brachial Artery Reactivity Task Force [37]. The measurement protocol has been described in detail previously [38]. Briefly, the diameter of the brachial artery and peak flow velocity were assessed by use of ultrasonography (350 Series, 7.5 MHz probe; Pie Medical) in combination with an arterial wall movement detection system (Wall Track System; Pie Medical). FMD was induced by placing a cuff on the forearm during 5 min with suprasystolic pressure (brachial systolic BP + 100 mmHg; where BP is blood pressure), resulting in an increase in blood flow, and subsequently increased shear stress after release of the cuff. Peak flow velocity was assessed within 15 s after release of the cuff, and the brachial diameter was measured after 45, 90, 180 and 300 s [31].

Endothelium-independent NMD (nitroglycerine-mediated vasodilatation) is considered to reflect smooth muscle cell function and is a control test for FMD. NMD was defined by measuring the diameter of the brachial artery and peak flow velocity 5 min after sublingual administration of nitroglycerine (400 μM; Nitrolingual Spray; Pohl-Boskamp) [31]. FMD and NMD were defined as the absolute change in brachial artery diameter and are expressed in mm.

IMT

IMT was measured with carotid ultrasonography as described previously [39]. Briefly, a single observer obtained structural properties of the right common carotid artery with an ultrasound scanner (350 Series; 7.5-MHz linear probe; Pie Medical). The scanner was connected to a personal computer equipped with wall track software (Pie Medical) that enables measurement of IMT (in mm) [39].

Other measurements

Fasting and 2-h post-load plasma glucose levels after a 75 g OGTT (oral glucose tolerance test) were measured in plasma and were used for classification into glucose tolerance categories [32]. Subjects who were already known to have diabetes or use glucose-lowering treatment were excluded from the OGTT. Brachial systolic and diastolic pressures were assessed in the left upper arm at 5-min intervals with an oscillometric device (BP-8800; Collin Press-Mate). Hypertension was defined as diastolic BP $\geqslant$ 90 mmHg, systolic BP $\geqslant$ 140 mmHg and/or using anti-hypertensive medication [40]. We also determined HbA_{1c} (glycated haemoglobin), fasting insulin, serum total cholesterol, HDL (high-density lipoprotein)-cholesterol, LDL (low-density lipoprotein)-cholesterol, triacylglycerols (triglycerides), serum creatinine, serum albumin, BMI (body mass index), waist and hip circumferences, smoking and prior cardiovascular disease, as described previously [3,39,41,42]. Insulin resistance was calculated using the HOMA (homoeostasis model assessment) formula (fasting insulin × fasting glucose/22.4) [43].

Statistical analyses

Population characteristics, expressed as means ± S.D., percentages or medians (interquartile range) in the case of a skewed distribution, were calculated according to the presence of any retinal abnormality. Differences were tested by means of an independent Student t test, χ^2 test or Mann–Whitney U test. Linear regression analyses were used to calculate the associations of cardiovascular risk factors, AVR, CRAE, CRVE, retinal SVC and retinopathy, which were regarded as independent variables, with FMD, NMD and IMT as the dependent variables. Associations were consequently adjusted for the stratification variables, age, sex and glucose-tolerance status. Additionally, regression models for FMD were adjusted for baseline diameter and increase in peak systolic velocity, and regression models for NMD were adjusted for baseline diameter. Next, associations were adjusted for potential confounders, such as cardiovascular risk factors. To make the results of linear regression models comparable among different independent variables, standardized β values are reported. A standardized β value of 0.1 indicates that, if the independent variable increases by one S.D., the dependent variable increases by 0.1 S.D. Effect modification by glucose tolerance status

Table 1 Clinical characteristics according to the presence or absence of any retinal abnormality

Values are means ± S.D., percentages or medians (interquartile range) in the case of skewed distribution. 'Any retinal abnormality' is defined as the presence of generalized retinal arteriolar narrowing, generalized retinal venular dilatation, retinal sclerotic vessel abnormalities or retinopathy. *P* values were calculated by an independent Student *t* test, Pearson's χ^2 test or Mann–Whitney *U* test. *Calculated according to the HOMA formula [fasting insulin (pmol/l) × fasting glucose (mmol/l)/22.4] [43].

	No retinal abnormality	Any retinal abnormality	*P* value
n (male/female)	123 (63/60)	132 (69/63)	
Age (years)	71 ± 7	72 ± 7	0.145
Diabetes (%)	43	46	0.595
HbA_{1c} (%)	6.2 ± 0.8	6.3 ± 1.0	0.125
BMI (kg/m^2)	27.3 ± 4.0	27.6 ± 4.2	0.485
Waist-to-hip ratio	0.93 ± 0.09	0.95 ± 0.10	0.112
Waist circumference (cm)	95.4 ± 9.9	97.3 ± 12.8	0.197
Hypertension (%)	74	83	0.154
Systolic BP (mmHg)	143 ± 18	151 ± 23	0.005
Diastolic BP (mmHg)	82 ± 10	86 ± 12	0.005
Total cholesterol (mmol/l)	5.8 ± 1.1	5.6 ± 0.9	0.064
HDL-cholesterol (mmol/l)	1.3 ± 0.4	1.4 ± 0.4	0.230
LDL-cholesterol (mmol/l)	3.7 ± 1.0	3.5 ± 0.8	0.038
Triacylglycerols (mmol/l)	1.4 (1.1–2.0)	1.4 (1.0–1.8)	0.154
Microalbuminuria (%)	13	23	0.116
Serum creatinine (μmol/l)	95 ± 15	99 ± 20	0.089
Fasting insulin (pmol/l)	63.0 (46.0–91.0)	67.0 (44.3–98.8)	0.441
Insulin resistance*	17.0 (11.9–30.0)	20.1 (12.0–33.3)	0.292
Current smoking (%)	11	12	0.920
Prior cardiovascular disease (%)	47	66	0.007
FMD (mm)	0.19 ± 0.22	0.16 ± 0.17	0.219
NMD (mm)	0.47 ± 0.23	0.45 ± 0.23	0.490
IMT (mm)	0.89 ± 0.18	0.91 ± 0.17	0.447
Generalized retinal arteriolar narrowing (%)		39	
Generalized retinal venular dilatation (%)		39	
Retinal sclerotic vessel abnormalities (%)		28	
Retinopathy (%)		24	

was investigated by entering a product term of glucose tolerance status multiplied by the predictor variable in the regression models. *P* values < 0.05 were considered as statistically significant. All analyses were performed in SPSS 12.0.1 for Windows 98.

RESULTS

Of the 256 participants, six had photographs that could not be graded for AVR and CRAE, and two had photographs that could not be graded for CRVE. FMD examinations were missing in 52 subjects, measurements of NMD in 64 subjects and measurements of IMT in 19 subjects, mainly because of poor definition of the arterial wall in the case of obesity [31]. The group with missing data had a significantly higher HbA_{1c} level, BMI and waist circumference (results not shown).

Baseline characteristics

Table 1 presents the clinical characteristics of the study population according to the presence of any retinal abnormality. Systolic and diastolic BP were significantly higher in individuals with any retinal abnormality compared with those without.

Retinal vascular disorders in relation with FMD and IMT

Table 2 shows the associations of AVR, CRAE, CRVE, retinal SVC and retinopathy with FMD and IMT after adjustment for age, sex and glucose-tolerance status, and for FMD, baseline diameter and increase in peak systolic velocity. Retinal vessel diameter and retinopathy were not significantly associated with FMD (Table 2). Retinal SCV had a weak significant association with FMD, which was attenuated and lost statistical significance after adjustment for NMD (standardized β value, 0.09; $P = 0.16$).

Table 2 Standardized β values for FMD and IMT associated with retinal microvascular disorders

Data are expressed as standardized β values (in mm) per change of one S.D. of the independent variable to facilitate direct comparison. Associations were adjusted for the stratification variables, age, sex and glucose tolerance status. Associations for FMD are additionally adjusted for baseline diameter and increase in peak systolic velocity. S.D. for FMD and IMT are 0.20 and 0.18 mm respectively.

		FMD		IMT	
Independent variables	S.D.	Standardized β (mm)	95 % CI	Standardized β (mm)	95 % CI
AVR	0.1	0.05	− 0.08 to 0.18	− 0.04	− 0.16 to 0.09
CRAE	18.9 μm	0.02	− 0.16 to 0.21	0.09	− 0.03 to 0.21
CRVE	27.1 μm	− 0.04	− 0.16 to 0.09	0.14	0.005 to 0.25
SVC	Yes compared with no	0.13	0.004 to 0.26	0.01	− 0.12 to 0.14
Retinopathy	Yes compared with no	0.05	− 0.08 to 0.18	0.02	− 0.11 to 0.15

Table 3 Adjusted standardized β values for IMT associated with CRVE

Values are expressed as standardized β values (in mm) per change of one S.D. of the independent variable to facilitate direct comparison. Similar results were obtained when adjusted for systolic and diastolic BP (as model 3), waist-to-hip ratio (as model 4), lipid profile (as model 5), serum creatinine (as model 6) or insulin resistance (as model 7).

		IMT	
Model	Added variables	Standardized β	95 % CI
1	CRVE, age, sex and glucose tolerance status	0.14	0.005 to 0.25
2	Model 1 + HbA_{1c}	0.12	0.002 to 0.24
3	Model 1 + hypertension	0.12	0.001 to 0.24
4	Model 1 + prior CVD	0.13	0.005 to 0.26
5	Model 1 + BMI	0.13	0.006 to 0.25
6	Model 1 + total cholesterol	0.13	0.003 to 0.25
7	Model 1 + cholesterol/HDL ratio	0.13	0.005 to 0.25
8	Model 1 + microalbuminuria	0.13	0.005 to 0.25
9	Model 1 + fasting insulin	0.08	− 0.04 to 0.21
10	Model 1 + smoking	0.12	0.004 to 0.24
11	Model 1 + hypertension, cholesterol/HDL ratio and smoking	0.03	− 0.01 to 0.24

In contrast with other retinal microvascular abnormalities, retinal venular dilatation was positively associated with IMT (Table 2). An increase of 27.1 μm (one S.D.) in CRVE was associated with an increase of 25 μm in IMT (0.14 S.D.). One-by-one adjustment for cardiovascular risk factors did not affect the association substantially, except for insulin, after which the association attenuated and lost statistical significance (Table 3). After multivariable adjustment for age, sex, glucose tolerance status, hypertension, cholesterol/HDL ratio and smoking, the standardized β value was 0.03 [95 % CI (confidence interval), − 0.01 to 0.24]. Associations did not statistically significantly differ over the glucose tolerance status categories ($P > 0.05$).

Additional analyses

After adjustment for age, sex, glucose tolerance status and baseline diameter, AVR, CRAE, CRVE, retinal SVC and retinopathy were not associated with NMD (results not shown). Exclusion of individuals using antihypertensive treatment did not materially change the associations between retinal vascular abnormalities and FMD, NMD and IMT (results not shown). In addition, generalized arteriolar narrowing and venular dilatation, defined as the lowest quintile of CRAE and the highest quintile of CRVE respectively, were not statistically significantly associated with FMD, NMD or IMT (results not shown).

DISCUSSION

This population-based study with elderly Caucasian subjects showed that retinal vessel diameters, retinal SVC and retinopathy were not significantly associated with impaired FMD. Retinal venular dilatation was positively related to increasing IMT, but adjustment for insulin explained a considerable part of this association. Taken together, the results of the present study do not provide strong support to the hypothesis that retinal microvascular abnormalities are markers of microvascular dysfunction that cause large artery endothelial dysfunction or early atherosclerosis.

The present study is the first observational study to investigate the relationship of retinal microvascular disease with endothelium-dependent FMD. FMD is an estimate of endothelial nitric oxide synthesis, which is an important property of endothelial function. Generalized endothelial dysfunction is considered a key factor in the pathogenesis of atherosclerosis [28,29]. Our results are in contrast with previous studies that have shown positive associations of plasma markers of endothelial dysfunction with AVR [23] and retinopathy [44,45]. However, expression of these markers and FMD may reflect different characteristics of endothelial function. Recently, Stehouwer et al. [27] reported a linear

association of microalbuminuria with impaired endothelium-dependent FMD and suggested that endothelial dysfunction might explain the relationship of microalbuminuria with CVD. If there is an association between retinal microvascular disease and macrovascular disease, as described previously [2,20–22,46], our present results do not support the hypothesis that large artery endothelial dysfunction is the causal link. There might be several explanations for our finding. First, the relationship between retinal microvascular disease and macrovascular disease may not be mediated by large artery endothelial dysfunction, but may have different pathways. Secondly, the retinal microcirculation may not sufficiently resemble the systemic microcirculation, which may still induce large artery endothelial dysfunction. Finally, the relationship between the subtle changes in retinal diameters and other retinal disorders with FMD and IMT may not be detectable due to a lack of power.

In the present study, we found a relationship between retinal venular dilatation and increased IMT, which is a marker of early atherosclerosis. This association was largely explained by insulin, which has been suggested to be an atherogenic factor [47,48] and has vasodilatory effects on vascular smooth muscle cells and/or endothelium [49]. The Rotterdam study reported, besides an association of retinal arteriolar narrowing with IMT, associations of larger venular diameters with carotid artery plaque and ankle-arm index [50], suggesting that not only narrowed retinal arterioles, but also larger retinal venules were associated with an adverse cardiovascular risk profile. However, there is no clear explanation for the associations of venular dilatation with atherosclerosis. Furthermore, the ARIC (Atherosclerosis Risk in Communities) study showed a relationship between retinal arteriolar narrowing with carotid artery plaque, but not with any other measure of atherosclerosis [23] and, in the CHS (Cardiovascular Health Study), retinal arteriolar narrowing was not associated with any early measure of atherosclerosis [24]. Taken together, considering the inconsistent results of previous studies, the results of our present study lend support to the hypothesis that retinal microvascular disease is not associated with subclinical atherosclerosis.

Strengths of our present study include extensive quantitative and qualitative measurements of retinal microvascular disorders from digital fundus photographs obtained in mydriasis in a population-based study. We used the revised formulas of Parr and Hubbard to quantify vessel calibre, by which the vessels are measured independently of image scale and the number of measured vessels [35]. In addition, FMD and IMT were measured by an experienced investigator [31,39]. The present study was performed in a population-based study with subjects with normal glucose metabolism, impaired glucose metabolism and Type II diabetes. However, results were similar regardless of glucose tolerance status.

The present study also had limitations. First, the large number of non-participants in the follow-up examination of the Hoorn Study may have implications for the interpretation of our results. The large proportion of non-participants may have limited the power, which reduces the precision of the estimates. In addition, as almost 20 % of the participants in the baseline examination had died at follow-up and the remaining non-participants had a higher baseline age, fasting glucose, HbA_{1c} level and more hypertension, we have to consider a healthy survivor effect and selection bias. In addition, the study participants with missing data had a significantly higher HbA_{1c} level, BMI and waist circumference. Therefore the loss of participants might be differential, thus the possibility of a relative underestimation of the true associations has to be taken into account. Secondly, the sample size of the follow-up study population is relatively small, in particular compared with earlier studies that used the computer-assisted method to measure retinal vessel diameters [11]. Therefore the effect size may be too small to reach statistical significance in our present study population. Thirdly, this population consists of subjects in an age range of 60–85 years, consequently with a relatively high prevalence of cardiovascular risk factors. Therefore generalizing the results of the present study to different populations, in particular of other age groups, has to be done with care. Finally, the measurement of FMD is based on several assumptions, e.g. that nitroglycerine entirely mimics the endogenous release of nitric oxide [51]. Nevertheless, there is increasing evidence that impaired endothelial-dependent vasodilatation, including FMD, is associated with an adverse cardiovascular outcome [52].

In conclusion, the present study shows that retinal microvascular disease was not independently associated with impaired FMD or increased IMT and may therefore be of limited value in risk stratification for future vascular events. Future prospective studies are needed to clarify this specific issue.

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